In a first experiment, about 10^5 NM-F9 cells stably expressing GM-CSF were cultivated in culture medium (see above) with and without fetal calf serum (fcs). After 1, 2, 3 and 4 days of cultivation without changing the medium the amount of secreted rhGM-CSF which accumulated over the time was determined by using the GM-CSF specific ELISA. About 14 ng GM-CSF was released by 10^5 cells in 4 days independently whether cultured with or without fcs (figure 7). The secretion rate after 1 day was slightly higher (about 6 ng/ml) when cultivated with fcs compared to the culture without fcs (about 4 ng/ml).

The human membrane protein Glycophorin A (GPA) was recombinantly expressed in NM-F9 cells as secretory Asialoglycophorin A using the expression vector construct secGPA. SecGPA contains the cDNA encoding for the N-terminal 91 amino acids (aa) of GPA without the transmembrane domain and the cytoplasmic tail. This cDNA was subcloned into the eukaryotic expression vectors pcDNA5/FRT/V5--His-TOPO and/or pcDNA3.1/myc-His C (both Invitrogen). Furthermore, NM-F9 cells were used to express recombinant fusion proteins of the secretory AGPA protein where the endogenous signal peptide was replaced by heterologous human signal peptides. Therefore, using molecular biological methods that are known to skilled persons the cDNAs secGM/GPA, secTCR/GPA and secAK/GPA were constructed where in place of the 19 N-terminal aa encoding cDNA the heterologous cDNA was integrated encoding for the signal peptides from GM-CSF (SEQ ID NO:1, MWLQSLLLLGTVACSIS, secGM/GPA), from the T cell receptor (SEQ ID NO:2, MACPGFLWALVISTCLEFSMA, secTCR/GPA) or from the antibody k light chain (SEQ ID NO:3, METDTLLLWVLLLWVPPGSTGD, secAK/GPA). The latter two signal peptides are already known to skilled persons for use as heterologous signal peptides to increase the secretory expression of any gene product. In case of secGM/GPA an additional alanine is expressed just between the signal peptide and the GPA encoding backbone. The three cDNA constructs were subcloned into the expression vectors mentioned above and the nucleotide sequences were confirmed. Each of the resulting expression vectors was used for the generation of stably transfected NM-F9 cells by using the procedure described above, electroporation, selection with hygromycin B and single cell cloning. In case of usage of the expression vector pcDNA5/FRT/V5-His-TOPO, genetically modified NM-F9 cells, which contained a lacZeo DNA cassette (Invitrogen) framed by two FRT recombinase recognition sites, were co-transfected with pcDNA5/FRT/V5-His-TOPO and the recombinase expression vector pOG44 (Invitrogen). secGM/GPA-, secTCR/GPA-, secAK/GPA- or secGPA-expressing cell clones were screened for secretion of a secretory AGPA that is released into the cell culture medium by a sandwich ELISA using the A83-CB12 antibody to catch any GPA out of the cell culture supernatant and the A63-C/A9 antibody that recognizes the TF-antigen localized on the extracellular domain. Figure 8 shows that the cell clones that produced the highest amounts of secretory AGPA (up to 40 ng per ml and per 10^5 cells) were generated with the secGM/GPA-construct. Using the other constructs maximally 13 ng secretory AGPA per ml and per 10^5 cells could be detected. Moreover, the number of cell clones that produced the secretory AGPA was significantly higher when the secGM/GPA-construct was used as expression vector (figure 8).